Expression of HCM causing mutations: lessons learnt from genotype-phenotype studies of the South African founder *MYH7*A797T mutation

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Genotype-phenotype correlations provide another perspective in studies seeking to identify the factors that underlie the clinical variability that is a feature of several inherited diseases. This approach has been particularly revealing in investigations into the molecular causes and phenotypic heterogeneity associated with hypertrophic cardiomyopathy (HCM), a common inherited primary cardiac disorder.1 2 Although, as its name suggests, hypertrophy may be a noticeable feature of the disease, it is not invariant, nor does the degree of hypertrophy necessarily correlate with the risk of sudden cardiac death (SCD), which is the most feared consequence of HCM.3 4

Molecular genetic investigations have shown that HCM is caused by more than 100 distinct mutations in at least seven different sarcomeric protein encoding genes.5 When the clinical features of HCM are correlated in a family context with the specific disease causing gene and its associated mutation, a recognisable pattern emerges. Essentially, mutations in the cardiac β myosin heavy chain gene (*MYH7*) are more often associated with echocardiographically detectable to marked hypertrophy and a variable risk of SCD, usually before the age of 35 years, which generally relates to the specific causative mutation.6 In striking contrast, mutations in the cardiac troponin T gene (*TNNT2*) are most frequently accompanied by subtle, or even undetectable, hypertrophy, yet confer a high risk of SCD in adolescence or young adulthood.3 4 Between these extremes lie the myosin binding protein C gene mutations, which are responsible for a considerably later age of onset of a steadily progressive form of hypertrophy, which may result in death from congestive heart failure later in life, more often than SCD.7 8 Further studies are needed before conclusive genotype-phenotype profiles can be defined for mutations in other HCM causing genes.9-11

This stratification of HCM into distinguishable subclasses of disease has been used in prognostication and management and counselling of patients with this treatable, but currently incurable, disease. However, it is generally advised that this approach be undertaken with caution, as some phenotypic associations are based on limited patient numbers.12 Furthermore, there is concern that data generated for one ethnic group cannot necessarily be extrapolated to others.13 As a corollary, evidence suggests that diverse genetic and environmental susceptibility factors may modulate the expression of identified disease causing mutations in the sarcomeric protein encoding genes.14 15

We previously described a novel *MYH7*mutation, resulting in the substitution of a threonine (T) residue for an alanine (A) residue at codon 797 (A797T), in two South African HCM patients. Initial impressions, based on the immediate family history of one of these unrelated subjects, were that this mutation was associated with a poor prognosis and frequent SCD, thus prompting further investigation.16 It was then found that both parents of the proband carried the *MYH7* A797T mutation. Concomitantly, this mutation was identified in another seven apparently unrelated members of a panel of South African HCM patients. Subsequent haplotyping studies showed that the *MYH7*A797T mutation was a founder mutation extant in South African subpopulation groups, where it accounted for 25% of disease causes in a panel of HCM affected probands,17

making it even more important to investigate its associated phenotype. These studies were further warranted because the mutation carriers in the extended families traced from these probands shared a common ancestor and provided a large patient base in which to establish genotype-phenotype correlations.

We show that the *MYH7* A797T mutation is generally associated with a favourable prognosis, but that it is important to consider the influence of both genetic and environmental modifiers on the disease profile, when using genotype-phenotype correlations in patient management and counselling.

The study subjects were probands belonging to a panel of South African HCM affected subjects and their relatives who could be traced. After obtaining informed consent from subjects or legal guardians, DNA was extracted from peripheral blood samples.18 The*MYH7* A797T mutation was detected by PCR based allele specific restriction enzyme analysis.16Mutation positive subjects were clinically evaluated as described previously.14 Briefly, echocardiographically determined end diastolic maximum left ventricular wall thickness (LVWT) measurements were obtained at the anterior interventricular septum (IVS) and posterior wall (PW). Echocardiographic diagnosis of HCM was made in the presence of an IVS $\geqslant$13 mm, in the absence of confounding factors. Electrocardiographic diagnosis of HCM was based on the presence of left ventricular hypertrophy (LVH) according to the point system of Romhilt and Estes19 or a significant Q wave abnormality. A family history and clinical records were obtained to identify the range of symptoms noted in affected subjects and the number of disease related and sudden cardiac deaths (SCDs) reported among the families studied. The latter data were used to construct Kaplan-Meier survival curves.20 Based on the similarity of phenotype and survival, the survival data from affected subjects in all the families, except those belonging to pedigree 101, were pooled. Pedigree 101 was evaluated separately because both parents in kindred 101a (fig 1) carried the*MYH7* A797T mutation and previous haplotype studies had shown them to be related.17 Members of the pedigrees are identified by a pedigree number prefix, followed by the subject's identification numbers. The prefix SB identifies probands from whom relatives could not be traced. Pedigrees 101, 124, 131, and 138 were of white descent, while pedigrees 104 and 110 and subjects SB902, SB983, and SB995 were of ethnic admixture.17 In order to ascertain that two brothers carrying the*MYH7* A797T mutation were monozygotic (MZ) twins, their *Hin*fI digested DNA samples were analysed by Southern blot hybridisation21 at hypervariable GTG genetic loci using the $(GTG)_5$ fingerprinting probe.22

A total of 66 family members of six pedigrees (pedigrees 101, 104, 110, 124, 131, and 138, fig 1) and three probands (SB902, SB983, and SB995) were genotyped for the *MYH7* A797T mutation. Of these, 39 were mutation carriers (table 1), including both parents (1.II.2 and 1.II.3) of the proband (1.III.6) of kindred 101a, within pedigree 101 (fig 1). Additionally, three dead sibs of 1.III.6, namely 1.III.1, 1.III.3, and 1.III.5, were diagnosed with HCM at necropsy, implying that they were likely to have inherited at least one copy of the mutant allele from one of their carrier parents. Of these subjects, 1.III.1 was definitely a heterozygous mutation carrier, as one of her fraternal twin offspring (1.IV.2) had inherited the mutation, while the other (1.IV.1) had not. Similarly, dead subject 1.III.3 could not have been a homozygous mutation carrier, as he also had one non-carrier child (1.IV.3). No further assumptions could be made about the carrier status of dead subject 1.III.5, who died childless. No living homozygous mutation carriers were detected in this branch of pedigree 101.

Thirty five of the 39 living mutation carriers were clinically examined, while 1.III.10, 4.II.3, 4.III.1, and 38.III.1 declined clinical investigation. Subjects 38.II.1, 38.II.6, and 38.III.3 were assessed only by ECG and 4.I.1, 4.II.1, and 10.I.1 only by echocardiography.

The mutation was associated with echocardiographically detectable hypertrophy of the IVS (LVWT $\geqslant$13 mm) in 17 subjects (table 1), and although not to the same extent, the left PW and/or apex were also abnormal, with measurements of greater than 11 mm, in 11 of these subjects (table 1). Additionally, there were three mutation carriers whose LVWT measurements were $\geqslant$11 but <13 mm. Moreover, SB902 and SB983 had undergone myectomy to reduce outflow tract obstruction, as well as mitral valve replacement. The mean LVWT, calculated for all mutation carriers $\geqslant$16 years, was 17.1 mm (SD 8.6), while the median LVWT was 13 mm.

Blood pressure measurements were below 160/95 mm Hg in 27 of the 29 mutation carriers for whom records were available (table 1), with the exceptions being two brothers, 31.III.6 and 31.III.8 (160/110 and 150/100 mm Hg, respectively). Only three of these 29 mutation carriers were receiving blood pressure lowering medication at the time of the initial examination, namely, 31.III.6, who suffered kidney disease, and 31.III.8 and 31.III.1.

Only four subjects had electrocardiographically detectable LVH, evaluated by the point system of Romhilt and Estes19; these subjects had also shown an LVWT of ⩾13 mm (table 1). Additionally, 1.III.6 had had a pacemaker inserted. A further 12 subjects had other ECG abnormalities often noted in HCM, while their LVWT values varied between 10 and 40 mm. Of the remaining 15 subjects who did not display any ECG abnormalities, nine also did not meet the echocardiographic diagnostic criterion, while two had not been investigated by echocardiography and three were less than 16 years old (table 1).

Disease penetrance among all mutation carriers was 61% (19/35 subjects), based on subjects older than 16 years for whom an LVWT of ⩾13 mm was measured (32 subjects, table 1) or who were diagnosed with HCM at necropsy (three subjects).

The symptomatic presentation of mutation carriers varied. Six of the 35 subjects for whom data were available complained of syncope or presyncope (1.II.2, 1.II.8, 1.III.6, 1.III.8, 31.III.4, SB902), three experienced dyspnoea (1.II.3, 1.III.8, 4.II.1), four suffered palpitations (1.II.8, 4.I.1, 31.III.1, 38.II.4), while six experienced both dyspnoea and palpitations (1.III.6, 1.IV.2, 10.II.1, SB902, SB983, SB995). Five subjects experienced angina (1.II.8, 1.III.6, 38.II.4, SB902, SB995), two suffered cardiac failure (1.II.2, 4.I.1), while three subjects developed both angina and cardiac failure (1.II.3, 4.II.1, SB983). However, further investigation of possible underlying ischaemic heart disease was not undertaken. Of the 35 clinically assessed mutation carriers, 19 subjects, of whom four were under 16 years of age, had no reported symptoms.

Proband 38.II.4 (fig 1) presented with chest pain and palpitations at the age of 38 years. He showed an LVWT of 12.7 mm at both the IVS and the left PW, and while his ECG tracings showed abnormal repolarisation and increased voltages (table 1), they did not meet the diagnostic criterion of LVH.19 However, taken together, the mild LVH, non-specific ECG changes, and symptoms, were likely to be HCM related. In contrast, his twin brother, 38.II.3, who was shown to be identical by band sharing on GTG fingerprinting, was symptom free, and exhibited an LVWT of 10 mm at the PW and 9 mm at the IVS. Although on ECG, 38.II.3 exhibited the same repolarisation abnormality and increased voltages as his twin, all other parameters, including similar blood pressure values, were normal. It was established from the proband's history that, while he had always been physically active, playing rugby football in high school and continuing to participate in competitive recreational cycling at the age of 38 years, his twin brother had generally avoided sport and exercise.

In kindred 101a, three of the four offspring of parents 1.II.2 and 1.II.3 had suffered SCD (fig 1). These events occurred in 1.III.1, 1.III.3, and 1.III.5 at 26 years, 24 years, and 17 years of age, while they were walking, playing tennis, and during a period of heightened emotion, respectively. Three of their mother's pregnancies had terminated in spontaneous abortions and she suffered SCD while sedentary at the age of 58 years. In contrast, no sudden, or disease related, deaths were known to have occurred in the recent generations of any of the other pedigrees, including the remainder of pedigree 101. In pedigree 131, one pregnancy in 31.II.3 had miscarried. The hypertensive father of the proband in pedigree 138 had died at 58 years of myocardial infarction, probably resulting from ischaemic heart disease. The Kaplan-Meier product limit curves for survival of the subjects with the *MYH7* A797T mutation belonging to pedigree 101 and that of the rest of the families with this mutation, as well as that of South African families carrying the R92W mutation in *TNNT2*,3 are shown in fig 2. While survival in the other pedigrees was unimpeded, survival in pedigree 101 was comparable to that of subjects bearing the*TNNT2* R92W mutation with its previously described poor prognosis.3

In this study, we have investigated the clinical phenotype associated with the HCM causative *MYH7* A797T mutation in 35 mutation carriers, of whom 32 were members of six different apparently unrelated

pedigrees, and three were from families who could not be traced. The present study shows that, although the initial impressions were to the contrary,16 the*MYH7* A797T mutation was generally associated with a good prognosis and normal life span, except in kindred 101a (figs 1 and 2).

However, despite the usually favourable prognosis, the mutation was generally associated with echocardiographically detectable to overt hypertrophy, which was often marked (table 1). Two mutation carriers, SB902 and SB983, had undergone septal myectomy, to reduce outflow tract obstruction resulting from their cardiac hypertrophy, as well as mitral valve replacement. The mean (SD) LVWT in all the pedigrees, excluding pedigree 101, was 17.8 (SD 8.7 mm), with a range of 8-40 mm for subjects over 16 years old (table 1). In pedigree 101, the mean (SD) LVWT was similar to these values, namely 16.6 (SD 9.6 mm), with a range of 9-36 mm, which was shown by ANOVA to have no statistically significant difference from the rest of the families with this mutation.

When considering survival, the only exception to the good prognosis associated with the *MYH7* A797T mutation was in pedigree 101. Here, the high frequency of SCD was restricted to kindred 101a (figs 1 and 2), in which haplotyping studies had previously indicated that the parents of the proband, both of whom carried the *MYH7* A797T mutation, shared a common ancestor.17 Three of the four offspring of the related mutation carrier parents in kindred 101a had died suddenly, before the age of 27 years. It is unlikely that these deaths resulted from a dosage effect of the mutant myosin protein, as at least two of them (1.III.1 and 1.III.3) could not have been homozygous mutation carriers by virtue of their non-carrier offspring (fig 1). In contrast to the poor prognosis observed in kindred 101a, the mutation was not associated with any disease related deaths in the rest of pedigree 101, nor in the other families carrying the *MYH7* A797T mutation (figs 1 and 2).

The data generated from genotype-phenotype assessments has led to speculation that HCM is not a simple monogenic disorder, as frequently the clinical manifestations and course of the disease differ even between subjects in the same family.14 15 For example, in the present study, there was marked variation in the degree of LVWT associated with the *MYH7* A797T mutation within members of the same pedigree (table 1), indicating that the phenotypic expression of HCM causing mutations is modulated by additional factors. Whether these factors are genetic or environmental, and what the nature of the interplay between these factors and the major HCM causing mutations are, has been a topic of discussion in recent papers.15 23 24

It can be speculated that the distinctive survival curve seen in pedigree 101 may result from the influence of genetic factors. In view of the shared ancestry of the parents in the nuclear family, it is possible that their offspring, who were deduced not to be*MYH7* A797T homozygotes, may have been homozygous for susceptibility alleles at other loci that play a role in modulating expression of the main disease causative gene. Further support for the influence of deleterious homozygosity is that while the proband, 1.III.6, the remaining living child of parents sharing a common ancestor, showed overt septal hypertrophy (36 mm), and had undergone pacemaker implantation, his half brother, 1.III.8, showed moderate hypertrophy (13 mm). However, as the degree of relatedness of the parents in kindred 101a could not be established, this proposal remains speculative. It is unlikely that environmental factors alone could account for the malignant phenotype seen only in kindred 101a, as the clinical course associated with the founder mutation was benign in all other affected families, whose individual members were exposed to varied environments and life style.

Evidence that environmental factors may also play a role in modulating the expression of main locus HCM causing mutations is provided by the divergent clinical presentation associated with the*MYH7* A797T mutation in the identical twin brothers in pedigree 138. Whereas, the physically active twin, 38.II.4, was symptomatic and showed an LVWT of 12.7 mm, with the hypertrophy appearing concentric and concentrated around the apex, the LVWT in his asymptomatic brother, 38.II.3, measured only 10 mm. Earlier studies have also documented heterogeneity in the clinical expression of HCM in MZ twins,24 25 but this is the first report of MZ twins genotyped for a specific HCM causing mutation, in which clinical differences may directly relate to exercise. The influence of physical activity on the expression of the hypertrophic phenotype in HCM has been a cause of speculation, as exercise is known to produce the hypertrophy seen in “athletes' heart”.26 However, although these twins were considered genetically identical, it cannot be excluded that

epigenetic factors, or indeed environmental influences other than exercise, could also have played a role in the dissimilar development of hypertrophy.

Currently, genotype-phenotype correlation studies provide the best available route to informed and accurate prognosis for improved patient management and risk stratification, with the caveat that these correlations should be based on substantial numbers of subjects drawn from the relevant ethnic group. In addition, it may also be prudent to consider the possible effects of shared ancestry and exercise on the clinical expression of a specific mutation, especially in subpopulation groups with known founder effects.

Although these examples of divergent phenotypic expression in kindred 101a and the twins in pedigree 138 are based on small numbers, they lend support to the notion that HCM is not a simple monogenic disorder and that both genetic and environmental factors are modifiers of the disease phenotype. A strategy followed in studies of disease phenotypes with multifactorial aetiology is to reduce the complexity of analysis by investigating genetically homogeneous subjects. The presence of the founder *MYH7* A797T mutation suggests that the families harbouring it share a degree of common ancestry. We therefore propose that the presence of this HCM causing mutation with incomplete penetrance, in a substantial group of related people, provides an opportunity to investigate the role of additional factors involved in the development of the disease phenotype. Only when these factors are known will the puzzling variability in the clinical expression which is a feature of HCM mutations, and the true pathophysiology of this disease, be understood.

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Table

Table 1
Echocardiographic and electrocardiographic features present in subjects carrying the A797T MYH7 mutation

Subject No		Sex/age (y)		Echocardiographic				Electrocardiographic		BP		SAM	Abn rep	Inc volts	Abn Q waves	↑Atria	LVH
				IVS (mm)	PW (mm)			Area of hypertrophy	ASH								
1.II.2	M/58	12	10	Ivs + apex		y	n	y	n	n	n	n	130/80				
1.II.3	F/55	22	10	Ivs		y	n	y	n	y	y	n	140/80				
1.II.6	F/53	9	8	NA		n	n	n	n	n	n	n	110/80				
1.II.8	F/47	14	9	Ivs		y	n	n	n	n	n	n	150/90				
1.III.6	M/34	36	13	Ivs + pw		y	y	PM	PM	PM	PM	PM	140/80				
1.III.8	M/29	13	13	Ivs + pw		n	n	y	n	n	n	n	120/70				

Subject No		Sex/age (y)		Echocardiographic				Electrocardiographic		BP		SAM	Abn rep	Inc volts	Abn Q waves	↑Atria	LVH
				IVS (mm)	PW (mm)			Area of hypertrophy	ASH								
1.III.10	M/30	NI	NI	NI		NI	NI	NI	NI	NI	NI	NI	NI				
1.III.11	F/27	10	8	NA		n	n	n	n	n	n	n	110/70				
1.IV.2	F/14	13	9	lvs		y	y	y	n	y	n	n	100/50				
1.IV.6	F/8	6	6	NA		n	n	n	n	n	n	n	na				
4.I.1	F/70	8	8	NA		NA	n	na	na	na	na	na	na				
4.II.1	M/48	24	12	lvs + pw		y	y	na	na	na	na	na	na				
4.II.3	M/na	NI	NI	NI		NI	NI	NI	NI	NI	NI	NI	NI				
4.II.6	F/33	9	9	NA		NA	n	n	n	n	n	n	120/80				
4.III.1	F/10	NI	NI	NI		NI	NI	NI	NI	NI	NI	NI	NI				
10.I.1	F/32	24	10	lvs		y	n	na	na	na	na	na	100/70				
24.II.1	M/56	28	16	lvs		y	y	y	y	n	n	n	150/80				
24.III.2	M/29	12	12	lvs + pw		NA	n	n	n	n	n	n	130/80				
31.III.1	M/52	10	10	NA		n	n	y	n	n	n	n	130/95				
31.III.4	M/51	18	11	lvs + apex		y	n	y	n	n	n	n	125/85				
31.III.6	M/57	13	12	lvs + apex + pw		n	n	y	n	y	n	n	160/110				
31.III.8	M/54	13	12	lvs + apex + pw		n	n	y	n	y	y	y	150/100				
31.IV.1	M/20	10	10	NA		n	n	y	n	n	n	n	130/80				
31.IV.2	M/18	9	10	NA		n	n	n	n	n	n	n	120/60				
31.IV.3	M/15	7	7	NA		NA	n	n	n	n	n	n	120/75				
31.IV.4	M/23	26	11	lvs + pw		n	y	y	n	n	n	y	130/60				

Subject No		Sex/age (y)		Echocardiographic				Electrocardiographic		BP		SAM	Abn rep	Inc volts	Abn Q waves	↑Atria	LVH
				IVS (mm)	PW (mm)			Area of hypertrophy	ASH								
31.IV.5	M/22	23	8	Ivs		n	y	n	n	n	n	n	120/70				
31.IV.6	M/19	40	na	Ivs + apex		y	y	y	n	n	n	n	110/70				
31.IV.8	F/15	10	10	NA		NA	n	n	n	n	n	n	140/90				
31.IV.14	M/27	9	9	NA		n	n	n	n	n	n	n	140/90				
38.II.1	M/40	na	na	na		na	na	n	n	n	n	n	na				
38.II.3	M/38	9	10	NA		NA	n	y	y	n	n	n	140/90				
38.II.4	M/38	12.7	12.7	Ivs + apex		n	n	y	y	n	n	n	145/90				
38.II.6	M/35	na	na	na		na	na	n	n	n	n	n	na				
38.III.1	F/12	NI	NI	NI		NI	NI	NI	NI	NI	NI	NI	NI				
38.III.3	F/13	na	na	na		na	na	n	n	n	n	n	na				
SB902	F/27	22[1]-150	13[1]-150	Ivs + pw		y	y	y	n	y	y	y	110/60				
SB983	F/62	16[1]-150	16[1]-150	Ivs + pw		y	y	y	n	y	y	y	140/70				
SB995	M/49	25	15	Ivs + pw		y	y	n	n	n	n	n	140/85				

- Abn Q waves = abnormal Q waves, Abn rep = abnormal repolarisation, Age = age at diagnosis, ASH = asymmetrical septal hypertrophy, ↑Atria = enlarged atria, BP = blood pressure (mm Hg), Inc volts = increased voltages, IVS (mm) = maximum end diastolic interventricular septal thickness in mm, Ivs = interventricular septum, LVH = left ventricular hypertrophy by Romhilt and Este's criteria, PW (mm) = maximum end diastolic posterior wall thickness in mm, pw = posterior left ventricular free wall, SAM = systolic anterior motion of the mitral valve, y = feature present, n = feature absent, NA = not applicable, na = records not available, NI = not investigated clinically,
- 1-150 = post myectomy values. Subjects under 16 years old are underlined.

Figures

Figure 1

Pedigrees in which the MYH7 A797T mutation segregated. The genotypic and phenotypic status of subjects is indicated in the key. Kindred 101a is shown on a shaded background.

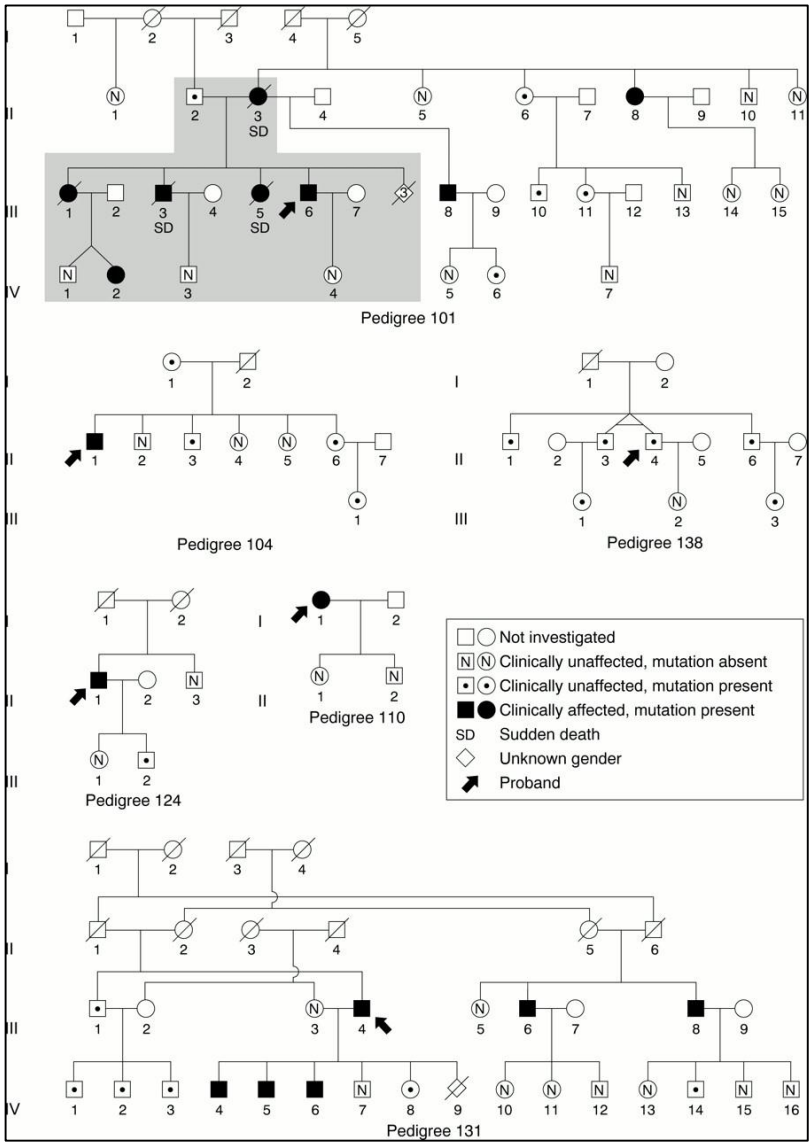
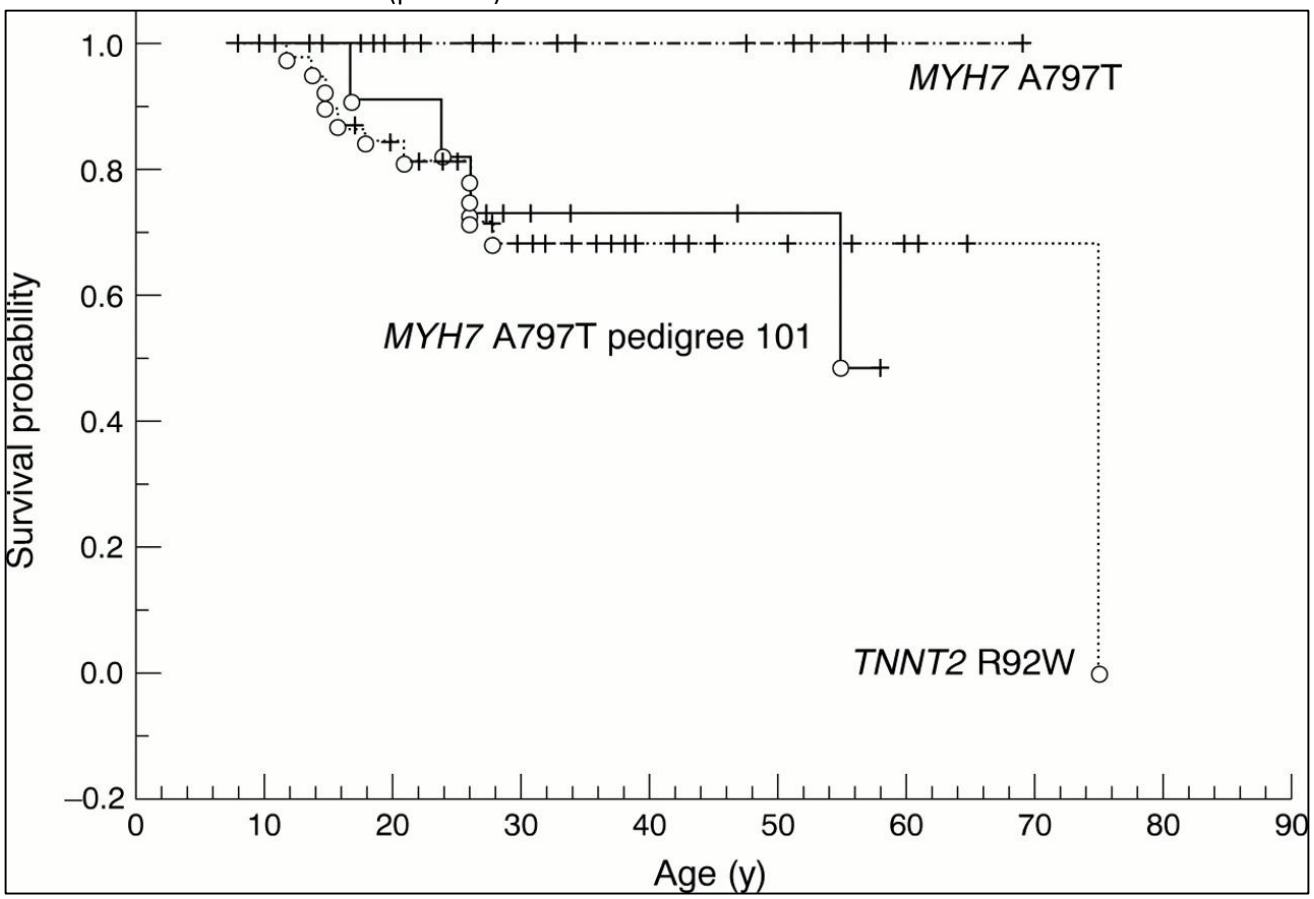


Figure 2

A comparison of Kaplan-Meier product limit curves for survival in subjects carrying the MYH7 A797T mutation and those carrying the previously reported TNNT2 R92W mutation.17 Survival data were combined and plotted for all MYH7 A797T families except for pedigree 101. Survival in pedigree 101 was similar to that of subjects with the TNNT2 R92W mutation (p=0.9), but was significantly worse than in the other families with the MYH7 A797T mutation (p=0.024).



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